Monitoring long-term efficacy of fampridine in gait-impaired patients with multiple sclerosis

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Abstract: Objective: To expand upon the limited knowledge of the long-term effects of prolonged-release (PR) fampridine in patients with multiple sclerosis (PwMS) regarding safety, walking improvements, and changes in drug responsiveness. Methods: Fifty-three PwMS who completed the FAMPKIN core study were included in this extension trial. Drug efficacy was assessed in an open-label and randomized double-blind, placebo-controlled study design with regular baseline assessments over a period of 2 years using the Timed 25-Foot Walk (T25FW), 6-Minute Walk Test (6MWT), and 12-item MS Walking Scale (MSWS-12) as outcome measures. Results: The data showed good tolerability and persisting efficacy of PR fampridine during long-term treatment in PwMS. Significant improvements in walking speed, endurance, and self-perceived ambulatory function were observed during open-label (T25FW: +11.5%; 6MWT: 10.7%; MSWS-12: 6.1 points) and double-blind controlled treatment with PR fampridine (T25FW: +13.1%; 6MWT: 11.9%; MSWS-12: 7.4 points). Several patients showed changes in drug responsiveness over time, resulting in an increased proportion of patients exceeding 10% or 20% improvements in walking measures after long-term treatment. Conclusions: Efficacy and tolerability data confirmed PR fampridine as a valuable long-term treatment for improving ambulatory function in gait-impaired PwMS. Similar results in open-label and double-blind phases reveal that the walking tests used are objective and reliable. The considerable proportion of patients in whom responsiveness to PR fampridine changed over time emphasizes the importance of regular reassessment of drug efficacy in clinical practice to optimize treatment. Such reassessments seem to be particularly important in patients with poor initial drug responses, as this group demonstrated enhanced responsiveness after long-term treatment.

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Author Contributions:

All coauthors have significantly contributed to the study and reviewed the manuscript. LF, BZ and ML planned and designed the extension trial. LF and BZ analyzed data, produced figures and prepared the manuscript. SK, KR, LL, TS, DW, TK, PG and JAP collected, analyzed and interpreted the data. LF and BZ performed the statistical analysis. MW and ML conceived and supervised the study and critically revised the manuscript. LF had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Conflict of Interest Disclosure:

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Abstract:

Objective:

To expand upon the limited knowledge of the long-term effects of prolonged-released (PR)-fampridine in patients with multiple sclerosis (PwMS) regarding safety, walking improvements and changes in drug responsiveness.

Methods:

Fifty-three PwMS who completed the FAMPKIN core study were included in this extension trial. Drug efficacy was assessed in an open-label and randomized double-blind, placebo-controlled study design with regular baseline assessments over a period of 2 years using the Timed 25-Foot Walk (T25FW), 6-Minute Walk Test (6MWT) and 12-item MS Walking Scale (MSWS-12) as outcome measures.

Results:

Data showed good tolerability and persisting efficacy of PR-fampridine during long-term treatment in PwMS. Significant improvements in walking speed, endurance and self-perceived ambulatory function were observed during open-label (T25FW: +11.5%; 6MWT: 10.7%; MSWS-12: 6.1 points) and double-blind controlled treatment with PR-fampridine (T25FW: +13.1%; 6MWT: 11.9%; MSWS-12: 7.4 points). Several patients showed changes in drug responsiveness over time, resulting in an increased proportion of patients exceeding 10% or 20% improvements in walking measures after long-term treatment.

Conclusion:

Efficacy and tolerability data confirmed PR-fampridine as a valuable long-term treatment for improving ambulatory function in gait-impaired PwMS. Similar results in open-label and double-blind phases reveal that the walking tests used are objective and reliable.

The considerable proportion of patients in whom responsiveness to PR-fampridine changed over time emphasizes the importance of regular re-assessment of drug efficacy in clinical practice to optimize treatment. Such re-assessments seem to be particularly important in patients with poor initial drug responses, as this group demonstrated enhanced responsiveness after long-term treatment.

Classification of evidence:

This study (NCT01576354) provides Class II evidence that PR-fampridine significantly improved gait compared to placebo in a 2 week study in patients with multiple sclerosis who had been using PR-fampridine for 2 years.

Introduction:

Multiple sclerosis (MS) is the leading neurological cause of persisting disability in young adults.[1]

Impaired mobility due to walking deficits occurs in approximately 75% of patients with MS (PwMS)[2, 3] and is reported by these patients as the single most devastating symptom.[4, 5]

Fampridine (4-aminopyridine, dalfampridine) is the only approved medication for the symptomatic treatment of gait disorders in PwMS in both the early and late phases of the disease.[6, 7] Prolonged-released (PR)-fampridine blocks voltage-gated potassium channels, thereby improving signal conduction in demyelinated nerve fibers.[8, 9] Whereas beneficial effects of short-term treatment with PR-fampridine on maximal walking speed [10-14] and additional functional outcomes [7, 13-15] (e.g. walking endurance) are well characterized in PwMS, little information exists regarding the long-term safety and efficacy of therapy with PR-fampridine. Open-label treatment over periods of 1 and 3 years demonstrated good tolerability and persisting improvement in ambulatory function[16] and fatigue.[7] Positive effects of PR-fampridine on dynamic stability and self-perceived walking ability were further demonstrated in a double-blind, placebo-controlled study over a period of 24 weeks.[15]

This study aims to expand upon the knowledge of the long-term effects of PR-fampridine on walking function in PwMS. In contrast to prior long-term studies on PR-fampridine, this study used annual drug holidays (open-label or placebo-controlled) to allow for accurate re-assessment of drug responsiveness, thus providing important information on changes in drug efficacy over time. Given the increasing number of

PwMS treated with PR-fampridine for MS-related walking dysfunction, there is a pressing need for a better understanding of the long-term effects of this therapy.

Methods:

Study population

This extension trial was performed at the University Hospital Zurich, Switzerland, between 2013 and 2015. All participants enrolled in the trial had previously completed the core study (FAMPKIN, clinicaltrials.gov; NCT01576354). Detailed inclusion and exclusion criteria of the core study are reported elsewhere.[14] No patients were excluded from the extension trial. In brief, participants diagnosed with relapsing-remitting, primary- or secondary-progressive MS with a clinically apparent walking impairment but able to cover a distance of at least 50 meters (with or without walking aids) in the 6-Minute Walk Test (6MWT) were included. Of 55 patients completing the core study, 53 (33 females; age: 50.3 ± 9.4 years; Table 1) were enrolled in the extension trial.

Standard protocol approvals, registrations, and patient consents

All experimental procedures of the study (NCT01576354) were performed according to the current guidelines of the Declaration of Helsinki and Good Clinical Practice and were approved by the Zurich cantonal ethics committee and the regulatory agency for medicines (Swissmedic). All participants provided informed, written consent to enter the extension trial.

Study design

A screening visit (S) was performed at least 2 weeks after completion of the FAMPKIN core study. Based on earlier reports,[11, 12, 14] a 14-day washout period is sufficient to avoid carry-over effects of PR-fampridine (Biogen, Cambridge, MA, USA). The first year of the extension trial was open-label, during which patients received 10mg PR-fampridine twice daily for 11.5 months (figure 1A). Long-term treatment with PR-fampridine was followed by a 14-day washout phase without treatment. Patients then received continuous, long-term treatment with PR-fampridine for a further 11.5 months before undergoing safety and efficacy assessments at the end of the second year in the form of a double-blind, placebo-controlled, cross-over component to obviate potential placebo effects. Analogous to the open-label drug holiday in the first year, the double-blind treatment periods were limited to 14 days. At V5, patients were randomized 1:1 into two groups (by the Zurich Cantonal Pharmacy), the first receiving placebo for 2 weeks followed by 2 weeks of PR-fampridine with the order reversed for the second group. All experimenters and participants were blinded to the treatment allocation at the time of data assessment. Allocation concealment was removed after the last patient had completed the double blind study visit (V7).

Study visits

Safety assessments comprising laboratory safety measures (hematology, blood chemistry, urine analysis, creatinine clearance), measurement of vital signs, reporting of adverse events (AEs and SAEs) and assessment of changes in concomitant medication were performed at each study visit (figure 1B). Outcome measures relating to treatment efficacy consisted of the Timed 25-Foot Walk (T25FW), the 6MWT and the 12-item MS

Walking Scale (MSWS-12) questionnaire and were assessed at S, V2, V3 and V5 (open-label) as well as at V6 and V7 (randomized, double-blind, placebo-controlled; figure 1B). The EDSS (Expanded Disability Status Scale) was assessed at S, V6 and V7. In addition to the MSWS-12, we also asked the patients to report on when they thought they were being treated with PR-fampridine or with placebo to investigate whether patients perceived fampridine-induced changes additional to ambulatory improvements.

Statistical analysis

Statistical analysis was performed with SPSS (V21, IBM Corp., USA). Three groups consisting of (1) all participants, (2) fampridine-responders and (3) non-responders were designated. Group classification was based on responder criteria defined in earlier publications [11, 12] and were applied based on the T25FW data of the core study: Fampridine-responders were participants achieving a faster walking speed in the T25FW for at least three of the four visits during the double-blind treatment periods compared to the maximum speed recorded during any of the five baseline visits. Chi square test and Fisher's exact test were used to assess statistical significance of categorical variables. Statistical analysis of continuous demographic data was performed using two-tailed, unpaired t-tests. Changes in walking performance during treatment with PR-fampridine or placebo were assessed by two-tailed, paired t-tests or repeated measures one-way ANOVAs.

Classification of evidence

The primary research question was to monitor the long-term effects of PR-fampridine on walking function in PwMS using three primary outcomes consisting of two quantitative clinical walking tests (T25FW, 6MWT) and the MSWS-12 questionnaire during open-label and randomized, double-blind, controlled assessments (Class II evidence).

Results:

Fifty-three PwMS (24 relapsing remitting; 5 primary progressive; 24 secondary progressive) with an EDSS between 3.0 and 7.0 (mean: 5.3 ± 1.2) were included in the extension trial (Table 1). Forty-seven patients completed the first year (30 females; age: 51.4 ± 9.3 years) and 36 patients (22 females; age: 52.0± 9.3 years) completed the second year of the trial. One patient was excluded from data analysis of V6 and V7 due to an elective surgical intervention between these visits. The demographics of the study population did not change over the 2 years of the extension trial (S, V3, V7; one-way ANOVA and two-tailed, unpaired t-test for age, disease duration, EDSS; Fisher's exact test for gender). The demographics of the randomized groups (PR-fampridine first vs. placebo first group) during the double-blind treatment phase were not different in terms of age, duration and type of MS, disability (EDSS), gender and concomitant treatment (Table e-1).

Long-term safety of PR-fampridine

Forty-five of 53 PwMS showed at least one AE during the extension trial, resulting in a total of 129 AEs (Table e-2). The most common AEs were urinary tract infections, nasopharyngitis and nervous system disorders (Table e-3). The degree of severity was

mild for most AEs (71%), moderate for a subset (28%) and severe for a single AE (erysipelas). The relation of AEs to treatment was reported as unlikely for 23% and as possible for 77%. The number of AEs did not differ between the double-blind placebo and PR-fampridine treatment phases (Table e-2; *p*=0.4913, Chi square test). Fourteen of the 53 participants experienced at least one SAE with a total of 21 SAEs; eight of moderate severity and 13 graded severe. While the majority of SAEs were unrelated to the study medication, a causal connection to the study medication was deemed unlikely in five SAEs and possible in one.

Long-term efficacy of PR-fampridine on walking function

Treatment with PR-fampridine resulted in improvements in walking speed (T25FW; *p*=0.0274, one-way ANOVA) and endurance (6MWT; *p*=0.0002) across all patients and visits (figure 2A). Effects of PR-fampridine in both subgroups (PR-fampridine responders and non-responders) were similar to the total population in terms of magnitude of effect, but only the improvement in 6MWT in non-responders was statistically significant. Walking speed and endurance worsened significantly after discontinuation of study treatment for 2 weeks at the end of the first year (V2 vs. V3: -11.5% in T25FW (95% confidence interval (CI) -4.9 to -18.0%); -10.7% in 6MWT (CI -7.3 to -14.0%)). In both clinical gait tests, patients substantially recovered their walking capacity on re-initiating open-label treatment with PR-fampridine for another 11.5 months (V3 vs. V5: +11.0% in T25FW (CI 3.5 to18.5%); +13.6 in 6MWT (CI 6.8 to 20.4%)). This treatment effect also remained stable during the double-blind, placebo-controlled assessment of drug efficacy: maximal walking speed improved by 13.1% (CI

4.1 to 22.1%) and walking endurance by 11.9% (CI 6.4 to 17.4%) during double-blind treatment with PR-fampridine compared to double-blind placebo treatment. Drug efficacy in the randomized group receiving PR-fampridine first was not different from that in the randomized group receiving placebo first during the double-blind treatment phase (p=0.2745 for T25FW, p=0.4339 for 6MWT, p=0.4313 for MSWS-12; unpaired, two-tailed t-test), indicating that the order of double-blind treatment did not influence drug efficacy. Overall walking capacity, as measured by the T25FW and 6MWT, worsened between the untreated baselines of the first year (figure 2A; S vs. V3), but remained constant between baselines at the end of the first and second year in our cohort (figure 2A; V3 vs. Plac). The averaged group performance while on PR-fampridine (V2, V5) remained at the level of the untreated baseline (S).
Paired, statistical analysis of drug efficacy during the open-label period (V2 vs. V3; figure 2B) and the double-blind controlled treatment phases (V6 vs. V7, figure 2B) revealed significant improvements during the open-label treatment in both functional tests and all sub-groups (except for non-responders in the T25FW; paired, two-tailed t-test). Similarly, efficacy of PR-fampridine during the randomized, double-blind phase was highly significant for the total population, but only reached statistical significance in the subgroup of non-responders in the 6MWT (p=0.0154).

Subjective long-term efficacy of PR-fampridine

Self-estimated walking function was improved by PR-fampridine (figure 2A; MSWS-12; p=0.0002 for all participants, one-way ANOVA). Patients showed worsened self-perception of ambulatory function when discontinuing the study medication for 2 weeks

(figure 2; V2 vs. V3: +6.2 points in MSWS-12 (CI 4.0 to 8.5 points); $p<0.0001$, paired, two-tailed t-test). The open-label effect of PR-fampridine on perceived walking function was highly significant for all subgroups (figure 2B). Drug efficacy during double-blind treatment was comparable to the open-label therapy in all participants (figure 2; V6 vs. V7: +7.0 points in MSWS-12 (CI -3.4 to -10.6 points); $p=0.0003$), but was not significant for the subgroups (figure 2B).

Twenty-two of 31 patients correctly identified their allocation to the PR-fampridine arm ($p=0.0196$; Chi square test; five patients declined to report).

Change in individual responsiveness to PR-fampridine over time

To accurately monitor long-term efficacy of PR-fampridine, we tracked the individual drug response in 33 patients who completed the FAMPKIN core study and 2 years of the extension trial (figure 3). Demographic data of the 33 patients did not differ from the total populations of the core study (55 patients) or the extension trial (53 patients). Longitudinal analysis of drug efficacy revealed that, in many participants, the response to PR-fampridine changed over time (figure 3A, B). Whereas overall drug efficacy as measured by the T25FW and 6MWT was not different after 2 years of PR-fampridine treatment compared to short-term treatment in the core study (figure 3C; T25FW: +3.4%, $p=0.3001$; 6MWT: +3.9%, $p=0.2136$), self-perceived walking function significantly improved over time (figure 3C; MSWS-12: -5.1 points, $p=0.0483$). To further assess patients' responsiveness to PR-fampridine, we analyzed the proportion of patients exceeding a specific threshold (T25FW and 6MWT: ≥ 10% improvement; MSWS-12: ≤ -6 points improvements) in the different tests during double-blind PR-

fampridine treatment of the core study and the double-blind treatment period in the extension trial. The proportion of patients improving more than 10% in walking speed was significantly higher during long-term treatment with PR-fampridine compared to short-term treatment (figure 4; p=0.0169; Fisher's exact test). Despite a similar trend in results, the proportion of patients responding in the extension trial versus the core study was not different in the 6MWT (short-term: 9/30 ≥ 10%; long-term: 15/30 ≥ 10%; p=0.1872) and the MSWS-12 (short-term: 10/33 ≤ -6 points; long-term: 12/33 ≤ -6 points; p=0.7944). While a majority of patients with substantially improved gait function on PR-fampridine in the core study also showed a similar response after 2 years of long-term treatment (figure 3 and 4), more than one third of patients exhibiting no or poor initial improvements with PR-fampridine in the core study changed their response to the drug and demonstrated improved walking function when tested again after 2 years (figure 4A). The trend towards increased responsiveness after long-term treatment was also observed for higher thresholds (i.e. 20% improvement in walking tests and 8 points in MSWS-12; figure 4B).

Fampridine-induced changes in clinical walking tests were not correlated with the self-assessed change in ambulatory performance in the double-blind core study (figure e-1; r=-0.2463, p=0.1742 for T25FW; r=0.0071, p=0.9705 for 6MWT). Conversely, in the double-blind phase of the extension trial, changes in MSWS-12 significantly correlated with clinical walking improvements (r=-0.4357, p=0.0127 for T25FW; r=-0.5851, p=0.0007 for 6MWT).

Discussion:

The results of this extension trial emphasize the good tolerability of PR-fampridine in PwMS supporting previously published safety profiles of long-term PR-fampridine treatment.[15-17] The proportion of patients experiencing AEs and SAEs was in line with previous reports.[15, 16] The frequency of AEs was not different between double-blind treatment with PR-fampridine and placebo, indicating that PR-fampridine was likely not responsible for the AEs in PwMS treated with the drug.

Our results demonstrate significant beneficial effects of long-term treatment with PR-fampridine in all tested clinical outcomes over 2 years. To prevent bias due to patients with a good drug response remaining in the trial and participants with low responsiveness dropping out, we only included patients who completed the core study and both years of the extension trial, allowing comparison of short- and long-term drug effects. Using different thresholds of effect size in the T25FW, 6MWT and MSWS-12, we demonstrated that a considerable proportion of participants changed their response to PR-fampridine over two years of long-term treatment. The proportion of participants reaching an improvement threshold of 10% increased from one fifth (T25FW) and barely one third (6MWT) to one half of all patients (T25FW and 6MWT) after 2 years of PR-fampridine treatment; a significant increase compared to short-term, double-blinded treatment. The majority of patients (80%) who showed greater than 10% improvement in the T25FW and 6MWT in the core study maintained a similar degree of responsiveness after long-term treatment. In contrast, 40% of patients initially falling short of the 10% improvement threshold in the T25FW and 6MWT during short-term treatment exhibited improvements ≥ 10% after 2 years. Increased responsiveness to PR-fampridine in these initial “poor responders”, combined with a potential decline of

drug efficacy in initial “good responders”, might explain the approximation of drug effects between responders and non-responders seen in earlier open-label long-term studies.[16] The observed changes in drug response during long-term treatment suggest that drug efficacy should be re-evaluated regularly, in particular in those patients who demonstrate a poor response to PR-fampridine after initial assessment. Discussion relating to the mechanisms underlying the observed changes in responsiveness to the study drug over time must remain, for the moment, speculative. Since MS is a dynamic disease with de- and remyelination and axonal and neuronal death contributing to ongoing, structural alterations of the CNS grey and white matter during its course,[18] responsiveness to PR-fampridine may likewise change over time. As a potassium channel blocker, it may initially improve signal conduction in demyelinated axons only to be rendered ineffective once these axons undergo degeneration with the natural course of the disease. On the other hand, appearance of new, demyelinating lesions may result in clinical deterioration amenable to improvement, at least partially and transiently, with PR-fampridine treatment at that stage. Enhanced drug efficacy over time may also be the result of training effects made possible by the improved neurological state induced by PR-fampridine. Finally, the development of pharmacological tolerance may result in a decrease in efficacy during long-term treatment. Identification of patient characteristics (e.g. type of MS, concomitant treatment) associated with changes in responsiveness to PR-fampridine over time should be the focus of future, larger trials.

The effect of PR-fampridine on self-perceived walking function reported here was higher than that reported by Huppert and colleagues[15], perhaps due to their shorter treatment duration of 24 weeks. More than one third of our patients reached a threshold of 6 and 8

points improvements in the MSWS-12 questionnaire after 2 years of treatment. Both thresholds are associated with clinically meaningful improvement of walking function.[15, 19, 20] Poor correlations between self-perceived walking function and clinical improvements during short-term treatment with PR-fampridine as observed in the FAMPKIN core study are in line with previous results.[19] Long-term treatment with PR-fampridine, in contrast, revealed significant correlations between changes in subjective ambulatory function and walking speed and endurance, implying that patients developed a better appreciation of the effects induced by PR-fampridine over time. Significant effects of PR-fampridine on MSWS-12 are underlined by patients' ability to correctly guess their allocation during double-blinded treatment arms.

The current trial used a mixed study design combining open-label assessment of drug efficacy representative of clinical practice, as well as a double-blind, placebo-controlled analysis of treatment effects allowing the objective assessment of changes in ambulatory function and self-perceived walking performance as a result of PR-fampridine. Similar drug efficacy during open-label and double-blind treatment phases emphasizes the objective and robust nature of the clinical walking tests used in this trial. Larger p-values for drug efficacy in the double-blind compared to the open-label phase are likely due to fewer patients completing the second year of the extension trial. Objective and reliable outcome measures of walking function are important in the translation to clinical practice, where patients are typically first assessed once before and again after 14 days of open-label treatment with PR-fampridine.[21]

In contrast to previous long-term studies,[15, 16] the present trial re-assessed drug efficacy at regular time intervals. The regular baseline assessments minimized the effect of

factors other than PR-fampridine which may influence walking function over time (e.g. disease progression, change in concomitant treatment), allowing close and accurate monitoring of drug responsiveness in individual participants over more than 2 years.

A key constraint of the current trial is the limited number of patients, which is due to the intensity and logistics of such a comprehensive study design required to accurately track patients' individual responsiveness over time. Further long-term data of PR-fampridine in PwMS are needed to confirm these results. A second limitation is that double-blind, long-term effects of PR-fampridine were only assessed in half of the participants, i.e. those receiving PR-fampridine at V5. The other half was randomized to be treated with placebo first before re-initiating treatment with PR-fampridine at V6. Treatment effects in both groups were, however, not different in any of the clinical walking tests.

This study demonstrates the good tolerability and significant persisting effects of long-term treatment with PR-fampridine on ambulatory performance in PwMS over a period of more than 2 years. PR-fampridine-induced walking improvements are perceived by most patients. Against a background of persisting improvements of walking function across all patients, longitudinal assessment of drug efficacy revealed remarkable changes in responsiveness to PR-fampridine in some patients. In particular, those patients who showed a moderate to poor response in terms of walking improvement under PR-fampridine at initial assessment were liable to improve their responsiveness to the drug over time, thus highlighting the necessity to regularly re-assess the efficacy of PR-fampridine on ambulatory function in PwMS. Persisting drug efficacy, combined with the fact that PR-fampridine has also demonstrated beneficial effects in chronic

progressive MS [14, 16], where alternative treatment options to improve walking function are virtually absent, confirm PR-fampridine as an important option in symptomatic, long-term treatment for gait-impaired PwMS.

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Figure legends

Figure 1 Experimental study design and schedule of assessments.

Figure 1. (A) Study design of the extension trial. The first 2 years of the extension trial were performed with an open-label format (S-V5). Thereafter, gait function was examined in two randomized, double-blind treatment periods of 2 weeks each, during which patients took either PR-fampridine or placebo in a cross-over component (V6, V7). **(B)** Schedule of study assessments. Abbreviations: EDSS: Expanded Disability Status Scale; T25FW: Timed 25-Foot Walk; 6MWT: 6-Minute Walk Test; MSWS12: Multiple Sclerosis Walking Scale (12-items); S: screening; V: visits.

Figure 2 Walking performance and self-perceived ambulatory function at different time points during the extension trial.

Figure 2. (A) Data is displayed relative to baseline performance at the screening visit (S). Assessments at V2 (long-term PR-fampridine), V3 (drug washout) and V5 (long-term PR-fampridine) were performed in an open-label study design. Measurements at V6 and V7 were performed under randomized, double-blind conditions. Data for the randomized cross-over visits V6 and V7 was allocated according to the respective treatment (placebo (Plac); PR-fampridine (Famp)). Results are shown for all participants (black), for PR-fampridine responders (red) and for non-responders (blue). Data points represent group mean values ± standard error of the mean (s.e.m). P-values indicate statistical significance (one-way ANOVA) of respective data curves. **(B)** P-values reflecting the effect of PR-fampridine on different clinical tests during the open-label treatment (upper table, V2 vs. V3) and during the randomized, double-blind, placebo-

controlled treatment phase (lower table, V6 vs. V7) for all patients (all), for PR-fampridine-responders (responders), and for non-responders. Only patients completing all treatment phases (V2 and V3; V6 and V7) were included in the analysis (two-tailed, paired t-test) Abbreviations: T25FW: Timed 25-Foot Walk; 6MWT: 6-Minute Walk Test; MSWS12: Multiple Sclerosis Walking Scale (12-items); V: visits.

Figure 3 Individual drug responsiveness during short- and long-term treatment with PR-fampridine.

Figure 3. (A) Subject-specific comparison of PR-fampridine effects during short-term double-blind treatment in the core study and long-term double-blind treatment in the extension trial. Data points represent treatment effects under PR-fampridine relative to placebo for single PR-fampridine responders (left) and non-responders (right) during the core study and the extension trial (T25FW: responders (n=10), non-responders (n=15); 6MWT: responders (n=10), non-responders (n=13); MSWS-12: responders (n=11), non-responders (n=15)). **(B)** Changes in drug responsiveness of single participants between the core study and the extension trial in the T25FW, 6MWT and MSWS-12. Fields are arranged according to the magnitude of change in responsiveness for each outcome (red: improved responsiveness after long-term compared to short-term treatment; blue: reduced responsiveness after long-term compared to short-term treatment). Patient ID number is indicated on the left side of each outcome (T25FW: n=32; 6MWT: n=30; MSWS-12: n=33; same for (C)). **(C)** Treatment efficacy during short-term double-blind treatment in the core study (white bars) and long-term double-blind treatment in the extension trial (black bars) over all patients. Abbreviations: T25FW: Timed 25-Foot Walk

Test; 6MWT: 6-Minute Walk Test; MSWS-12: 12-item Multiple Sclerosis Walking Scale; Resp: PR-fampridine responders, Non-Resp: non-responders, *: $p<0.05$.

Figure 4 Changes in drug responsiveness over time

Figure 4. (A) Proportion of patients improving in walking speed (+10%), endurance (+10%) and self-perceived walking ability (-6 points) during the double-blind, controlled treatment phases in the FAMPKIN core study and the extension trial. The group of patients improving more than 10% in clinical walking tests or more than 6 points in the MSWS-12 is indicated in blue (pie chart), whereas patients showing decreased responsiveness to PR-fampridine are displayed in red. **(B)** As in figure 4A, but using higher threshold values (walking speed and endurance: 20%; self-perceived walking ability: 8 points). Only patients who completed the second year of the extension trial were included in the analysis (T25FW: n=32, 6MWT: n=30, MSWS-12: n=33). Abbreviations: T25FW: Timed 25-Foot Walk Test; 6MWT: 6-Minute Walk Test; MSWS-12: 12-item Multiple Sclerosis Walking Scale; pts: points.

Table 1 Demographic data of the study population at screening.

Table 1. Abbreviations: DMF: dimethyl fumarate; EDSS: Expanded Disability Status Scale; PPMS: primary progressive MS; RRMS: relapsing-remitting MS; SPMS: secondary progressive MS; SD: standard deviation; y: years.

Table 1

		screening	1st year of extension		2nd year of extension	
		Included (n=53)	Completed (n=47)	Discontinued (n=6)	Completed (n=36)	Discontinued (n=11)
Age (y), mean ± SD		50.3 ± 9.4	51.4 ± 9.3	48.4 ± 8.0	52.0 ± 9.3	50.3 ± 9.4
Gender, number (%)	Male	20 (38)	17 (36)	3 (50)	14 (39)	3 (27)
	Female	33 (62)	30 (64)	3 (50)	22 (61)	8 (73)
Type of MS, number (%)	RRMS	24 (45)	20 (42)	3 (50)	16 (44)	4 (36)
	PPMS	5 (10)	5 (11)	0	4 (11)	1 (9)
	SPMS	24 (45)	22 (47)	3 (50)	(16 (44)	(6 (55)
Disease duration (y) from diagnosis, mean ± SD		12.7 ± 7.3	13.7 ± 7.5	12.3 ± 5.0	15.4 ± 8.1	10.9 ± 5.4
EDSS, mean ± SD	No treatment	5.3 ± 1.2		6.3 ± 0.3		5.7 ± 1.1
	Placebo				5.1 ± 1.3	
	PR-fampridine				5.0 ± 1.3	
Concomitant MS treatment, number (%)	Total	29 (55)	25 (53)	4 (67)	17 (47)	7 (64)
	Interferon	7 (13)	5 (11)	1 (17)	3 (8)	2 (18)
	Natalizumab	17 (32)	14 (30)	2 (33)	6(17)	4 (36)
	Fingolimod	5 (9)	5 (11)	1 (17)	4 (11)	1 (9)
	DMF	0	1 (2)	0	4 (11)	0